I hereby certify that, on the date shown below, this correspondence is being transmitted via the Patent Electronic Filing System (EFS) addressed to Examiner Dana H. Shin at the U.S. Patent and Trademark Office.

Date: January 19, 2010

Ann E. Rabe, Reg. No. 56,697

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Glauco P. Tocchini-Valentini, *et al.*
Serial No.:	10/821,777
Filed:	April 9, 2004
For:	METHOD OF RNA CLEAVAGE AND RECOMBINATION
Group Art Unit:	1635
Examiner:	Dana H. Shin
Attorney Ref.:	911076.90023
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DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner For Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Glauco P. Tocchini-Valentini, on oath say and declare that:

1. I am the same Glauco P. Tocchini-Valentini who is a named inventor of the above-identified patent application. Since 1998, I have been the Scientific Director of the A. Buzzati-Traverso Campus, Monterotondo (Italy, Rome). As the Director, I supervised the following institutions: European Molecular Biology Laboratory (EMBL) outstation on mouse genetics; International Centre for Genetic Engineering and Biotechnology (ICGEB) outstation on mouse genetics; European Mouse Mutant Archive (EMMA); CNR Institute for Cell Biology. I have studied RNA since 1962 and was a post-doctoral fellow with E. Peter Geiduschek, Francis Crick and Sydney Brenner. I have been a Professor of Molecular Genetics both at the University of Chicago and the University of Rome. A copy of my *Curriculum Vitae* is attached as Exhibit A. In April 2009 I was elected foreign associate of the National Academy of Sciences of the United States of America. My work on RNA cleavage and recombination obtained a specific recognition.

2. I have reviewed the July 17, 2009 Office Action issued by the US Patent and Trademark Office. I understand that Claims 1 and 4-17 are rejected as being obvious over Abelson, et al., (The Journal of Biological Chemistry, 1998,273:12685-12688) in view of Diener et al. (Molecular Cell, 1998, 1:883-894) and Reyes et al. (Analytical Chemistry, 1987, 166:90-106). I submit this Declaration to provide evidence explaining that neither Abelson nor the other references cited provide the knowledge or motivation to a skilled person that eukaryal tRNA endonucleases could cleave trans-formed BHBs in non-tRNA target RNA molecules.

3. The Examiner alleges that Abelson teaches that tRNA endonuclease inherently cleaves the BHB structure of a tRNA substrate and that Reyes teaches cleaving an artificially-synthesized, non-tRNA oligonucleotide substrate that contains the BHB motif, each containing the G/A and U/A dinucleotides. The Examiner argues that one of skill would be motivated to do so because Abelson teaches that the BHB-motif structure-based cleavage of a target RNA oligonucleotide substrate is a conserved, absolute biological mechanism of a tRNA splicing endonuclease which can cleave any "universal" substrate containing the BHB motif, and Reyes teaches that tRNA splicing endonuclease-mediated tRNA cleavage was demonstrated to occur in any synthetic oligonucleotide substrate having proper structures that can be recognized by the tRNA splicing endonuclease. I disagree.

4. Abelson actually teaches that "it is likely that what has been conserved since the divergence of the Eukary and the Archaea is the endonuclease active site and the means to array two of them in a precise and conserved spatial orientation." (Abelson, p. 12688, last paragraph). Abelson cites as support for this "the results of Tocchini-Valentini and co-workers", specifically, Fabbri et al., Science 280 (1998) 284-286, "where it is demonstrated that both the eukaryl and archaeal endonucleases can accurately cleave a universal substrate containing the BHB motif." (Abelson, p. 12688, last full paragraph). Accordingly, when the Examiner cites Abelson as teaching that the BHB-motif structure-based splicing or cleavage of a target RNA oligonucleotide substrate is a conserved, absolute biological mechanism of a tRNA splicing endonuclease which can cleave the "universal" BHB substrate, she is actually citing Fabbri.

5. Fabbri *et al.* lists eight (8) authors that are as follows: Stefania Fabbri, Paolo Fruscoloni, Emanuela Bufardeci, Elisa Di Nocola Negri, Maria Baldi, Domenica Gandini Attardi, Emilio Mattoccia, Glauco P. Tocchini-Valentini. I was the principal author of Fabbri *et al.* and drafted a majority of the manuscript.

6. At that time of Fabbri *et al.*, we were not at all interested in producing trans-formed BHBs. At the time of Fabbri, it was neither known, nor predictable that a trans-formed BHB could result in cleavage of an RNA molecule by eukaryal tRNA endonucleases. While the exciting possibility that different cis-formed BHB-like motifs that could function as substrates (other RNA substrates in my sentence) for some tRNA splicing endonucleases could exist in nature was discussed, but it was not known or at all predictable.

7. In addition, FIG. 1 of Fabbri *et al.* showed cleavage of pre-tRNAs having the following structures: (1) a 5'-terminal phosphate group; (2) an acceptor stem comprising a seven base pair stem made by the base pairing of the 5'-terminal nucleotides with the 3'-terminal nucleotides; (3) a CCA tail at the 3' end; (4) a D loop comprising a four base pair stem ending in a loop; (5) an anticodon loop comprising a five base pair stem whose loop contains the anticodon; and (6) a T loop comprising a five base pair stem. The pre-tRNAs were cis-formed and structurally distinct from those shown in the application by having the structures noted above.

8. FIG. 2 showed cis-formed mini-substrates, which are also distinct from those shown in the application by having a 6 nucleotide loop that is not present in the structures shown in the application). In RNA hairpins, like the cis-formed mini-substrates shown in Fabbri *et al.*, the sequence of the loop is essential for the formation and stability of the structure. The fact that cis-formed mini-BHBs were cleaved by eukaryal endonucleases does not immediately implicate that the skilled person could form an active trans-formed structure and predict that an eukaryal endonuclease would subsequently cleave the trans-formed structure. In fact, it was not until 2001 that my group started to consider trans-formed structures and non-tRNA molecules as substrates for eukaryal endonucleases (*see, e.g.*, Fruscoloni P, *et al.*, EMBO Reports 2:217-221 (2001); attached as Exhibit B).

9. Furthermore, we found in subsequent years that even more relaxed forms of BHB are cleaved by enzymes like the one derived from *Sulfolobus sulfataricus* (*see*, Tocchini-Valentini G, *et al.*, PNAS, 102:8933-8938 (2005); Tocchini-Valentini G, *et al.*, PNAS, 102:15418-15422 (2005); and Tocchini-Valentini G, *et al.*, PNAS, 104:12300-12305 (2007); all attached as Exhibit C).

10. Accordingly, it is my opinion that one of ordinary skill in the art would understand that Abelson does not contemplate or disclose to one of ordinary skill in the art that a trans-formed BHB motif (lacking a terminal loop) could be successfully cleaved by eukaryal tRNA endonucleases (see, e.g., FIGS. 4 and 13 of the application for transformed BHBs). Thus, the claimed methods of my invention do not require all the structures present in pre-tRNA for cleavage, which allows one to advantageously cleave non-tRNA molecules.

11. It was neither known, nor predictable, at the time of the Abelson or Fabbri references that a trans-formed BHB could result in cleavage of an RNA molecule by eukaryal tRNA endonucleases. In fact, the claimed methods of my invention are directed toward my surprising finding that such enzymes can recognize and cleave trans-formed structures having only a BHB motif. It is my opinion that neither Fabbri, nor Abelson, teach or suggest the surprising finding of my invention: that a trans-formed BHB could result in cleavage of an RNA molecule by eukaryal tRNA endonucleases and that such enzymes can recognize and cleave trans-formed structures having only a BHB motif.

12. I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements are made with knowledge that willful false statements, and the like so made, are punishable by fine or imprisonment, or both, under Section 1001, Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated this 13th day of January 2010

Glauco P. Tocchini-Valentini